STUDIES ON DRUG INDUCED CONDITIONED TASTE AVERSION

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ABBREVIATIONS

CS	conditioned stimulus
CTA	conditioned taste aversion
H_A	alternate hypothesis
H_O	null hypothesis
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
M	molar
SPR	saccharin preference ratio
UCS	unconditioned stimulus

CHAPTER I

INTRODUCTION

Conditioned taste aversion (CTA) is the learned avoidance of a substance when its ingestion is followed one or more times by the administration of certain pharmacological agents or physical treatments. The terminology of classical (or Pavlovian) conditioning designates the aversive agent or treatment as the unconditioned stimulus (UCS). The conditioned stimulus (CS) is the substance (actually the taste of the substance) which is subsequently avoided.

Though it is possible to use a CS with which the subject is familiar (Ahlers & Best, 1971; Jacquet, 1973), it is more common practice to expose the subject to a novel stimulus. Saccharin has been widely used as CS due to its distinctive taste and the ease with which subjects form associations to it. Although saccharin has little or no nutritional value, it is not a neutral stimulus. The highly rewarding properties of this substance are well documented (Sheffield & Roby, 1950; Collier, 1962; Foster, 1968; Colavita, 1968). Saccharin solution availability results in a marked increase in the fluid consumption of mice or rats once a subject's initial neophobia to a novel taste is overcome (Garcia, Hankins, & Rusiniak, 1974).

When rats are given simultaneous access to both tap water and saccharin solution, the consumption of saccharin solution accounts for up to 90% of total fluid consumption in subjects which have not been conditioned to avoid saccharin (Martin & Ellinwood, 1973). Thus, the establishment of a CTA to saccharin requires the learning of an aversion to a stimulus which would otherwise be rewarding. This property of the CS is important in establishing criteria for the attainment of a CTA response. In what is referred to as a "single-bottle" test (only the CS solution is available for drinking), the consumption of saccharin after aversive conditioning is compared to the consumption prior to conditioning, or to an unconditioned control subject's consumption on test day. Such a comparison must acknowledge the changing baseline of consumption in control animals. Subject's initial consumption (on treatment or "pairing" trials) will be attenuated by neophobia for the novel solution. Subsequent consumption will rise with repeated presentations of the CS until the full reward potential of the CS is apparent (Garcia et al., 1966; Garcia & Ervin, 1968; Berman & Cannon, 1974).

An alternate test which avoids the problem of a changing baseline is the "two-bottle" choice test. In this paradigm the CS is again the only available solution during pairing with the UCS drug or treatment, but when

subsequent aversion is tested both the CS and tap water are available. From the record of the relative consumption of the two solutions a "saccharin preference ratio" score is computed (saccharin preference ratio = [saccharin consumption in g or ml/total fluid consumption in g or ml] X 100). A score of 100% indicates that only saccharin was consumed, a score of 50% indicates equal consumption of saccharin and water, and a score of 0% indicates that only water has been consumed. The saccharin preference ratio of a treatment group may then be compared to the ratio of an untreated control group, which is usually about 90% (Martin & Ellinwood, 1973). The two-bottle test is reportedly a more sensitive test for aversion (Grote & Brown, 1971), but typically results in a somewhat higher heterogeneity of variance (Martin & Ellinwood, 1973). It has also been reported that preference scores in two-bottle tests are markedly influenced by prior experience with the CS (Goudie & Thornton, 1975), hence the one-bottle test may be more appropriate when many training sessions are used. The advantage of a two-bottle test is that because the subject's preference is measured, the overall motivation to drink cannot confound the results.

Despite the common terminology, CTA differs from classical conditioning in several respects. First, a single CS-UCS pairing is effective for many drugs (Berger,

1972; Carroll & Smith, 1974; Nachman & Hartley, 1975). Secondly, the time interval between presentation of the CS and administration of the UCS may be much longer (several hours) than would be effective in classical conditioning (Garcia et al., 1966; Garcia & Ervin, 1968; Berger, 1972; Cappell & LeBlanc, 1975a). That such an extended CS-UCS interval should be effective is in direct violation of the "contiguity rule" of conditioning which specifies that punishing or rewarding stimuli must immediately follow signals, or responses, or both, if learning is to occur. Thirdly, the Pavlovian rule of the "equipotentiality of conditioned stimuli (CS)," which holds that any perceptible stimulus can signal an animal that reward or punishment is eminent, is apparently also violated by CTA learning. A series of studies (Garcia et al., 1966; Garcia & Ervin, 1968, Garcia et al., 1974; Rozin, 1969) have shown that subjects acquire aversions only to the taste of the food or solution, not for the size or shape of the food, nor for the dish it is served in, nor for a clicking sound produced electronically by licking the drinking spout. The preferential association of taste with UCS effects has been termed the "belongingness hypothesis." The same series of reports found that, if punished by electric shock, a rat will acquire an aversion to the clicking sound, but it is very difficult to establish a taste aversion to electric shock.

From these results Garcia et al. (1968, 1974) concluded that when animals suffer a "general malaise" of the type presumably produced by CTA training they display avoidance responses to chemical (gustatory or olfactory) stimuli but not to telereceptive (auditory, visual, or tactile) stimuli. When peripheral pain is the UCS, the converse is true. As supporting evidence for such a dual neural control system they noted that gustatory and visceral systems, including the area postrema, send afferent fibers directly to the nucleus of the fasciculus solitarius. Telereceptive and cutaneous systems do not.

Properties of the UCS Agents

Since most of the original CTA studies used X-irradiation, apomorphine, or lithium chloride as the UCS, it was first thought that the mechanism by which drugs or physical treatments induce CTA involved gastrointestinal distress. It now seems doubtful that such distress is necessary for establishment of a CTA response.

Berger, Wise, and Stein (1973) investigated the effects of lesions in the area postrema on taste aversions. This site has been classified as an emetic chemoreceptor trigger zone and its ablation reduces drug-induced vomiting in cats (Borison & Wang, 1953). In rats, such lesions prevented formation of a CTA based on methylscopolamine but not CTA based on amphetamine, indicating that the integrity

of the area postrema is not necessary for establishing CTA to amphetamine. In another study (Levy et al., 1974) pre-treatment with an anti-emetic was ineffective in preventing CTA formation. In general, the evidence suggests that neither emesis, nausea, gastrointestinal malaise, nor "sickness" in general are necessary to induce CTA (Berger, 1972; Gamzu, 1974; Cappell & LeBlanc, 1977; Vogel, 1976). In fact, "sickness" may not even be sufficient to induce CTA. Nachman and Hartley (1975) used UCS drugs and dosages which were chosen for their toxicity (doses of rodenticides sufficient to produce behaviorally observable malaise). Sodium fluoroacetate (1.6 mg/kg), copper sulfate (5.0 mg/kg), red squill (35.0 mg/kg), thallium sulfate (10.0 mg/kg), and lithium chloride (127.2 mg/kg; which is not a rodenticide) were effective UCS agents after only one pairing with saccharin. Yet doses of warfarin (12.5 mg/kg), cyanide (2.0 mg/kg), and strychnine (1.0 mg/kg) which were 30 to 50% of the LD_{100} were insufficient to produce CTA; additionally, a near lethal dose of strychnine (2.0 mg/kg) was only marginally effective. A comparable study reports the failure of cyanide (4.0 mg/kg), pyrollopyrimidine (15.0 mg/kg), gallamine (40 mg/kg), and malonate (0.1 M; 1% body weight) to produce conditioned taste aversions (Ionescu & Buresova, 1977). The investigators emphasized the poor correlation between the degree of observable malaise due to the drugs and their relative effectiveness in CTA production. Lithium

chloride produces only a mild diarrhea but is quite potent in CTA production. Strychnine, however, produced death by convulsion in several subjects without producing more than a marginal CTA to saccharin in survivors.

The range of effective UCS drugs is extremely wide. Lithium chloride (Cannon et al., 1975), apomorphine (Gamzu, 1975; Wise et al., 1976), amphetamine (Berger et al., 1973; Cappell & LeBlanc, 1971), fenfluramine (Goudie & Thornton, 1975), scopolamine and methylscopolamine (Berger et al., 1973), barbiturates (Vogel & Nathan, 1975), ethanol (Cannon et al., 1975), morphine (Coussens et al., 1973; Parker, Failor, & Weidman, 1973; Cappell et al., 1975), diazepam (Gamzu, 1975), meprobamate (Gamzu, 1975), mescaline (Cappell & LeBlanc, 1971), delta-9 tetrahydrocannabinol and hashish extracts (Ellsmore, 1972; Corcoran, 1973), and even glucose (Deutsch et al., 1976) are among the drugs reported effective as the UCS in the CTA paradigm. Physical treatments which are effective as the UCS include X-irradiation (Garcia & Koelling, 1967) and rapid whole body rotation (Braveman, 1977).

The only pharmacologically active compounds which have been reported as ineffective UCS drugs by more than one author are strychnine (Berger, 1972; Nachman & Hartley, 1975; Vogel, 1976), cyanide (Nachman & Hartley, 1975; Ionescu & Buresova, 1976), and cocaine (Cappell & LeBlanc, 1975b & 1977). It may yet prove that these "ineffective"

drugs are capable of producing CTA when administered in high enough doses. It is now known that cocaine, for example, must be administered at a dose of 10 to 36 mg/kg to obtain even a very weak CTA (Goudie et al., 1978), and that such a dose produces marked behavioral effects. However, the need for such high doses remains in contrast to most of the effective UCS drugs. Amphetamines can induce CTA at a dose (0.1 mg/kg) which has minimal behavioral effects (D'Mello et al., 1977). Goudie et al. (1978) suggest that the difference between these two compounds may be due to their different temporal profiles of action. Peak behavioral effects occur much sooner for cocaine (15 min.) as opposed to d-amphetamine (one hour). Cocaine also has a shorter duration of action. It is interesting that while cocaine is a weak aversive agent for CTA it is a very potent reinforcing agent in self-administration studies even at doses below 0.64 mg/kg i.v. (Thompson & Pickens, 1975).

It is difficult to explain how such a wide variety of agents of different pharmacological classes could all be effective through a gastrointestinal (GI) mechanism since many have no well established GI side effects and a few of the effective UCS drugs are actually used to relieve certain types of GI distress (anticholinergics: atropine and scopolamine). The range of effective UCS drugs is also not explained by the general toxicity of high doses. A number of authors (Berger, 1972; Cappel et al., 1973;

Cappell & LeBlanc, 1977; Vogel, 1976) have produced CTA using psychopharmacological agents at doses which do not produce any signs of toxicity. Indeed, some drugs such as amphetamine (Pickens & Harris, 1968), morphine (Weeks, 1962), and apomorphine (Baxter et al., 1974) are self-administered by experimental animals at dosages which are effective in establishing CTA.

It appears, in fact, that some agents can be aversive and rewarding at the same time. Wise et al. (1976) demonstrated that subjects would lever-press for the very injections that produced taste aversions. First it was demonstrated that rats with a history of d-amphetamine self-administration (1.0 mg/kg i.v.) could learn an aversion for the same dose and vice versa. In a second experiment animals were trained to lever press for amphetamine, then apomorphine (0.5 mg/kg i.v.) was substituted. The intent of the authors was to present the saccharin CS on the first day in which the reinforcing effect of the drug could be demonstrated. Previous work showed that when animals having a history of amphetamine self-administration are switched to apomorphine, the rewarding properties of apomorphine are apparent on the first day of substitution. Immediately prior to the switch to apomorphine, the amphetamine-trained animals were presented with a novel saccharin solution. On the following day, those subjects whose rate of responding indicated stable apomorphine

self-administration were again given access to saccharin. These subjects showed an aversion relative to controls in a one-bottle test. Apomorphine self-administration sessions were continued to verify that lever pressing was truly sustained by apomorphine rather than by the habit established under amphetamine.

Physiological Manipulation of CTA

Another possible explanation for the wide variety of effective agents would be that all the effective UCS drugs affect some common physiological system or act through a common biochemical substrate. The CNS would appear to be a likely candidate since many of the agents (chlordiazepoxide, diazepam, barbiturates, amphetamines, and hallucinogens) do have CNS effects. Yet, some of the active drugs (methylscopolamine, methyl atropine, or copper sulfate, for example) do not readily penetrate the blood-brain barrier. To hypothesize a common physiological system or substrate one must explain how both drugs of an agonist-antagonist pair can be effective in producing CTA. Vogel (1976) produced CTA with both methyl atropine and eserine, which have opposite effects on cholinergic systems. Similarly, both chlorpromazine and apomorphine, which have opposite effects on dopaminergic systems, are effective inducers of CTA (Gamzu, 1977). Treatments that increase histamine levels are effective (Levy et al., 1974)

and so are drugs with some antihistaminic effects (chlorpromazine: Berger, 1972). Finally, both morphine and its antagonist naloxone can be used to establish CTA (LeBlanc & Cappell, 1975).

In view of the above findings it would seem that if there exists a single common aversion-inducing mechanism it is one which is relatively non-specific with respect to the UCS drugs and physical treatments employed. One hypothesis is that certain stress-related physiological changes which are produced by the various treatments form the generalized aversive property common to all the different UCS drugs and treatments (Fischer, 1978; Braveman, 1977). Noting that systemic histamine levels peak one to two hours after X-irradiation, Levy et al. (1974) demonstrated that pretreatment with an antihistamine could block the formation of radiation-induced CTA. Such pretreatment was ineffective, however, against CTA induced by lithium chloride, and was only partially effective in blocking the aversion induced by cyclophosphamide (Levy et al., 1974; Levy, 1975).

Another line of research into the stress hypothesis is centered upon the pituitary-adrenal system. The evidence suggests that adrenocorticotropic hormone (ACTH) from the anterior pituitary may facilitate the acquisition of passive avoidance behavior (Levine & Jones, 1965; Guth et al., 1971) while suppression of ACTH release

through the administration of exogenous corticosterone (resulting in decreased ACTH release due to negative feedback) interferes with avoidance behavior (Levine & Levin, 1970; Bohus, 1973). These results were obtained in passive avoidance tasks in which an animal learns to avoid that portion of the cage in which electric shocks are delivered. Hennessy et al. (1976) extended these findings to the study of CTA. Injections of lithium chloride in a dose suitable for producing CTA (126 mg/kg) resulted in a sustained increase in plasma corticosterone levels. In the second part of their experiment, animals were pretreated with either 400 µg of dexamethasone (a synthetic glucocorticoid), saline, or ACTH (8.0 IU) given access to a flavored solution, and then given a lithium chloride injection. The results suggest the ACTH pretreated group established an aversion somewhat stronger than that of the saline pretreated controls, while the dexamethasone pretreated animals had a somewhat weaker CTA than did the controls.

Results of a recent study (Ader et al., 1978) indicate that the adrenal gland itself need not be intact for conditioning to be effective. Bilateral adrenalectomies were performed on 45 male rats and confirmed by post-experiment determination of plasma corticosterone concentrations. Control subjects were sham-operated. Experimental animals were maintained in a healthy state by

providing a 0.9% saline solution for drinking and by administering daily injections of dexamethasone (2.0 µg) in sesame oil. Control animals continued drinking tap water and were injected with sesame oil only. After recovery from sugery and adaptation to a restricted drinking schedule the subjects were given access to saccharin (saccharin in saline for experimental animals) and then injected i.p. with 50 mg/kg cyclophosphamide. Control groups for both the adrenalectomized and sham-operated animals had the UCS drug paired with tap water or saline as the CS. Two days after this single CS-UCS pairing, subjects were tested for CTA formation using a single-bottle test. Adrenalectomized animals had a significantly higher baseline intake of saccharin on pairing day (attenuated neophobia) but, taking this factor into account, did not differ from controls in the volume of saccharin consumed on test days.

The Pretreatment Effect

Another line of evidence which suggests a relatively non-specific aversion-inducing mechanism(s) comes from studies of the effect of pre-exposure to the UCS drug or treatment prior to CTA conditioning. Subjects pretreated with the UCS drug show less aversion to the flavored solution (CS) after pairing with the UCS than do saline pretreated controls. In some cases they show no aversion at all (scores comparable to an untreated animal). The

pretreatment effect also has been demonstrated for a wide variety of drugs and treatments: ethanol (Berman & Cannon, 1974; Cannon et al., 1975), amphetamine (Cappell & LeBlanc, 1975a; Goudie & Thornton, 1975; Goudie et al., 1976), chlordiazepoxide (Cappell & LeBlanc, 1973), barbiturates (Vogel & Nathan, 1976), apomorphine (Gamzu, 1975), morphine (Parker et al., 1973; Jacquet, 1973; LeBlanc & Cappell, 1974), and lithium chloride (Cannon et al., 1975).

An early demonstration of the pre-exposure effect developed from an attempt to condition a taste preference to a CS. Such results had only been attained when a subject suffering thiamine deficiency was exposed to a novel taste (CS) followed by an injection of thiamine (UCS), or when the CS was presented as the subject recovered from apomorphine induced illness (Garcia et al., 1974). Parker et al. (1973) wished to determine if comparable results could be obtained by alleviating a need that does not occur in nature and for which the species could not have evolved repletion mechanisms. Morphine withdrawal, they reasoned, should produce such an "artificial need state" in animals dependent on morphine, thus pairing morphine with the first presentation of the CS should produce a preference for the CS under these conditions. Rats were pretreated for 25 days with a dose of morphine which began at 20 mg/kg and increased by 5 mg/kg daily until a final dose of 140 mg/kg was obtained. This was followed by a three day period in

which morphine was withheld. Initial preference for a sucrose-octa-acetate solution was established during this 96 hour withdrawal period using a continuous two-bottle preference test (this procedure constituted a pre-exposure to the CS before conditioning as well as UCS pre-exposure). Beginning on day 30, a three day cycle was instituted in which the taste of sucrose-octa-acetate was paired with 95 mg/kg morphine, followed by two days of limited access to plain tap water with no drug injections. This three day cycle was repeated seven times (seven "pairings" or conditioning trials) and then preference on a two-bottle test was measured over a 96 hour period. Control groups included one which received the pretreatments but not the conditioning trials (morphone paired with no liquid instead) and a second which received saline instead of morphine during pretreatments (non-dependent control) but had the morphine-taste pairings. Prior to conditioning, both of the morphine pretreated groups were more aversive to the flavor than saline pretreated controls. This effect was observed daily during the 96 hour withdrawal period, indicating that withdrawal stress may be an effective UCS (may be associated with a novel taste). After conditioning the group which received both the morphine pretreatments and the CTA pairings showed an attenuated CTA relative to the two control groups, which did not differ. CTA in the

experimental group was attenuated with respect to preconditioning level of aversion (preference shift) as well as in comparison to controls. Parker et al. (1973) concluded that the pretreatment effect (attenuation of CTA) was due to the conversion of the UCS to a positively reinforcing stimulus in subjects having an artificially induced need state. The identical preference of the two control groups appeared to be due to morphine withdrawal on the one hand and to morphine presentation (to a non-dependent animal) on the other.

In a different paradigm Jacquet (1973) maintained mice on morphine (25-250 mg/kg escalating over 10 days, or constant doses in this range in other replications) or saline, and paired each with water or with saccharin 15 times. Each trial was both a CS-UCS pairing and a one-bottle test. Experimental subjects showed a progressive CTA to either solution over days. A behavioral test for dependence (compulsive jumping during withdrawal or after treatment with 25 mg/kg naloxone) indicated that the animals treated with morphine were dependent but did not have a conditioned preference or even an attenuated aversion to the CS. When the combined pairing and test trials were given at 5 days intervals at constant doses (to produce tolerance, but not dependence), the same results were obtained. Jacquet concluded that the aversion obtained was due to morphine pairing rather than morphine abstinence.

The degree of aversion was dependent on dose and number of pairings but was independent of injection environment.

Morphine was again one of the UCS drugs used by LeBlanc and Cappell (1974) in investigating the pretreatment effect. Prior to restricting drinking schedules, rats were pretreated with: a) 20-200 mg/kg morphine escalating over 16 days, b) saline for 11 days followed by 20-40 mg/kg morphine, dosage escalated over 5 days, or c) saline for 16 days. The UCS dose of morphine was 20 mg/kg and was paired with saccharin six times and each injection was given 42 hours after the last pretreatment or pairing dose. The two dependent groups received either the 20 mg/kg morphine or saline as UCS and had supplemental doses of morphine to maintain their dependence. The saline pretreated group was also divided and given additional saline injections. Morphine pretreatment in this manner was totally effective in blocking CTA. In the dependent groups conditioned to saline an upward trend occurred in saccharin consumption even though saccharin was repeatedly paired with 41 hours of morphine withdrawal. This finding is inconsistent with the Parker et al. (1973) study and LeBlanc and Cappell (1974) suggest the previous data may have reflected an unconditioned neophobia rather than an aversion based on withdrawal. LeBlanc and Cappell replicated their study using amphetamine (pretreatment schedule of 10-20 mg/kg or 2-4 mg/kg escalated over 15 days; and 1.0 mg/kg

UCS dose on pairing days). Results were the same except that amphetamine abstinence did not have the tendency to disrupt fluid intake, whereas morphine abstinence did. LeBlanc and Cappell (1974) suggest that pretreatment induces tolerance to morphine or amphetamine which renders the UCS drug less effective. They reject an explanation based on dependence and withdrawal since morphine and amphetamine differ greatly in ability to produce withdrawal symptoms (Kalant et al., 1971).

To investigate the rate of acquisition and loss of the pretreatment effect, Cappell and LeBlanc (1975b) pretreated rats with a constant (7.5 mg/kg) dose of amphetamine for a total of 0, 1, 5, or 20 days prior to CTA training with a 1.0 mg/kg dose of the drug. Results indicated that 5 or more pretreatments were needed at this dose to attenuate CTA. The time lapse between the end of pretreatments and the beginning of CTA training was also investigated. Subjects were withdrawn from a 20 mg/kg pretreatment regimen of amphetamine for 1, 7, or 14 days prior to CTA conditioning. The UCS pairing dose of amphetamine was 1.0 mg/kg. The group which had received no pretreatments for 14 days prior to training did not have an attenuated aversion, indicating that pairing trials must follow pretreatments within seven days if the pretreatments are to be effective. Elkins (1974) also investigated the effect of number of pretreatments. Six

pretreatments with cyclophosphamide (12.5 mg/kg) attenuated CTA induced by the same dose and increased the rate of extinction of the aversion. Three pretreatments failed to attenuate CTA magnitude but did reduce resistance to extinction. One pretreatment had no effect.

Another series of studies which manipulated many of the experimental parameters known to be involved in CTA was conducted by Cannon et al. (1975). When the UCS was 400 mg/kg ethanol administered intragastrically, pretreatment for five, three, or even only one day attenuated CTA development by the same dose and the effect was greatest for the greatest number of pretreatments. To investigate the role of tolerance a 0.02 ml/g body wt. dose of 0.12 M lithium chloride was chosen as UCS (delivered intragastrically, as were all UCS and pretreatment drugs). Lithium chloride was chosen because it is not known to produce metabolic tolerance. Four groups received either a single lithium chloride pretreatment or a single saline injection, eight, four, or one day prior to conditioning. Conditioning consisted of eight CS-UCS pairings using the same dose of LiCl or saline. These trials were also test days, since consumption was measured in a one-bottle test. The aversive animals showed a progressive decline in saccharin intake over the eight days. Only the group pretreated one day prior to the eight conditioning trials had an attenuated CTA. These results agree with Jacquet (1973)

concerning the effectiveness of a single pretreatment but are in contrast to Cappell and LeBlanc's (1975a) and Elkins' (1974) data which indicated the necessity of at least three pretreatments. The demonstration of the pretreatment effect for lithium chloride led Cannon et al. (1975) to argue that while tolerance may well be a factor in the pretreatment effect it is not a necessary prerequisite for demonstrating the attenuation of CTA.

In another part of this study (Cannon et al., 1975) the same dose of lithium chloride was paired with a novel flavor (Sustecal liquid diet) or with water. Twenty-four hours later, conditioning an aversion to saccharin was attempted. The group having UCS pretreatment paired with liquid diet was less aversive to saccharin than the group having UCS pretreatment paired with water. It appears that preceding a UCS pre-exposure with an explicit cue (liquid diet flavor) reduces the degree to which pretreatment is effective in attenuating CTA to a different taste CS. This result is incompatible with the "novelty hypothesis," which holds that any two groups having prior experience with the UCS (reduced novelty of the UCS induced drug state) should have the same degree of attenuation of CTA to that UCS (Gamzu, 1974, 1975, & 1977). Likewise, a group given pretreatments of the lithium chloride UCS, but no pairing to the CS, failed to develop a preference for saccharin over and above that of a group never given

the UCS. This result is incompatible with the hypothesis that the CS is associated with UCS withdrawal, as postulated by Parker et al. (1973).

When Cannon et al. (1975) varied the dose of lithium chloride used in pretreatment and/or pairing (0.02 ml/g body wt. of 0.0, 0.12, or 0.36 M lithium chloride) they reported that each pretreated group had an attenuated CTA relative to the naive group paired with the same dose. When the pairing (conditioning) dose was held constant a greater pretreatment dose produced less aversion. When pretreatment dose was held constant a greater pairing dose produced greater aversion to saccharin. Finally, it was demonstrated that no difference in the level of CTA could be obtained by pretreating some groups in an environment different from others. This observation does not support the theory that subjects make associations to other possible stimuli in the injection environment which then compete with saccharin for the role of CS when aversions are being conditioned (association hypothesis).

The Crossover Pretreatment Effect

Tolerance and/or the reduction of drug novelty have often been postulated as explanations for the pretreatment effect (Cappell et al., 1973 & 1975; Jacquet, 1973; Berman & Cannon, 1974; Gamzu, 1974, 1975, & 1977). Yet there remains a line of evidence which these relatively simple

concepts also have difficulty explaining. Pretreatment with one drug or procedure may attenuate CTA production by a totally different compound. This phenomenon has been termed the "crossover pretreatment effect."

An early demonstration of the crossover pretreatment effect employed morphine and amphetamine as UCS drugs (Cappel et al., 1975). Amphetamine pretreatment (10-20 mg/kg increased over 13 days) attenuated CTA production by both amphetamine (1.0 mg/kg) and morphine (6.0 mg/kg). However, morphine pretreatment (5-40 mg/kg, increased over 14 days) was not equally effective for both drugs. The morphine pretreatment attenuated CTA induced by morphine (6.0 mg/kg) only, and was not effective in attenuating CTA induced by amphetamine (1.0 mg/kg).

Goudie and Thornton (1975) used constant doses of amphetamine (2.0 mg/kg) and fenfluramine (6.0 mg/kg) in a crossover pretreatment design. Fenfluramine was chosen because it does not produce dependence and is not self-administered by rats. Subjects received nine pretreatments of either amphetamine, fenfluramine, or saline, followed by a single pairing to one of the two drugs or to saline. The level of aversion was determined using a one-bottle test. Results showed that the doses used were sufficient to produce CTA when saline pretreatments were given. However, fenfluramine appeared to produce a somewhat stronger aversion than did amphetamine. Pretreatment with either drug attenuated CTA induced by the same

compound. In the crossover groups it was found that fenfluramine pretreatment attenuated CTA induced by amphetamine but not vice versa. Like the results of the Cappell et al. (1975) study, the results of this work indicated an asymmetry in the ability of the two drug pretreatments to attenuate CTA.

Asymmetrical results were also obtained by Vogel and Nathan (1976) when three pretreatments of amobarbital (120 mg/kg), d-amphetamine (2.0 mg/kg) or saline preceded pairing of saccharin to one of these three agents. The drug doses used produced equivalent levels of CTA when pretreatments were with saline. Neither drug was capable of attenuating CTA produced by amphetamine at these doses. Amobarbital pretreatment did attenuate amobarbital-induced CTA however, and amphetamine pretreatment partially attenuated amobarbital-induced CTA as well.

Gamzu (1977) reported that three pretreatments with 2 mg/kg amphetamine attenuated CTA induced by the same dose of amphetamine, or CTA induced by a 15 mg/kg dose of chlordiazepoxide. Three pretreatments with 15 mg/kg chlordiazepoxide attenuated CTA to the same dose of chlordiazepoxide only, but had no effect on CTA induced by amphetamine (2.0 mg/kg).

Goudie et al. (1976) reported that pretreatment with d,l-methamphetamine (3.0 mg/kg) attenuated CTA produced by the same dose and that the extent of CTA was inversely

proportional to the number of pretreatments given. The effect reached an asymptote after nine pretreatments (further increases in the number of pretreatments did not increase the attenuation of CTA). When 14 pretreatments of the 3 mg/kg dose of methamphetamine were given, the attenuation of a 10 mg/kg pairing dose of methamphetamine was much less pronounced than that associated with the 3 mg/kg pairing dose. The 14 pretreatments with methamphetamine (3.0 mg/kg) failed to attenuate CTA induced by fenfluramine (5.0 mg/kg), chloramphetamine (5.0 mg/kg) or morphine (20 mg/kg). These results indicated the importance of choice of pairing doses, pretreatment doses, and duration of pretreatment. The authors noted that it is difficult to state unequivocally that pretreatment with drug X fails to attenuate CTA induced by drug Y, since a different dose or number of pretreatments might prove effective.

Cannon et al. (1977) compared the degree of attenuation due to pretreatment with the same drug (pretreatment effect) with the attenuation due to pretreatment with a different drug (crossover pretreatment effect). Subjects were pretreated four times using a gastric intubation procedure. After a three day period in which no drugs were given, the animals were placed on a conditioning schedule in which CS-UCS pairing occurred every third day. A total of three CS-UCS pairings were used. The UCS drugs and

doses used were 500 mg/kg ethanol (37.5% v/v) and 0.02 ml/g body wt. of 0.10 M lithium chloride. Pretreatments of ethanol, LiCl, or saline were matched with each of the agents in turn as the CS-UCS pairing drug. The ethanol and LiCl doses were equivalent in ability to produce CTA in saline pretreated controls. The attenuation of CTA due to pretreatment with the same drug used for pairing (pretreatment effect) was greater than the attenuation due to pretreatment with a different drug (crossover pretreatment effect). All pretreatments except saline were effective and the crossover pretreatment effects were symmetrical. A behavioral measure of tolerance (rotarod performance test) indicated a correlation between the level of CTA achieved and the degree of tolerance observed only for the groups pretreated with the same drug used for pairing. The authors conclude that some sort of general effects are operative in both types of pretreatment. When pretreatment is with the same drug, additional drug-specific effects such as tolerance are also operative.

Crossover effects between drug treatments and physical treatments have been reported by Braveman (1977). Five pretreatments of d-amphetamine (2.0 mg/kg), methylscopolamine (1.0 mg/kg), or a 10 mg/kg injection of 0.3 M LiCl all effectively reduced CTA induced by whole body rotation for 15 minutes at 60 rpm. This study has often been cited as evidence that the crossover effect is not tied to the

pharmacological nature of the drug treatments. This conclusion is compatible with the hypothesis of Cannon et al. (1977) that drug-specific effects are operative only when pretreatment drugs and pairing drugs are identical.

The common finding that crossover effects are asymmetrical may be due to parametric problems. The finding of Goudie and Thornton (1975) that fenfluramine pretreatent attenuated CTA induced by amphetamine, but not vice versa, led these investigators to study the effect of duration of action of a drug. Fenfluramine has a longer duration of action than amphetamine and appears to induce a stronger CTA in the absence of drug pretreatments. Thus, when fenfluramine was the pairing drug, it may have been more difficult to demonstrate a pretreatment effect since the degree of aversion was stronger to begin with. Goudie and Thornton (1977) tested the effect of prolonging the duration of action of amphetamine by inhibition of drug metabolism with SKF 525A. Rats were injected with saline or SKF 525A (10 mg/kg) two hours before the pairing of saccharin with saline or a 0.5 mg/kg dose of amphetamine. When saline was the UCS pairing agent the saline pretreated and SKF pretreated groups did not differ, indicating that pretreatment per se did not affect fluid intake. When amphetamine was the pairing drug the saline pretreated group displayed a typical aversion, while the SKF pretreated group displayed an even greater aversion. Apparently the

aversive properties of amphetamine were potentiated by the SKF pretreatment, presumably through prolonging the duration of action of amphetamine.

Goudie and Dickins (1978) again demonstrated the importance of duration of action, dose, and number of pairing trials in a study using nitrous oxide as the UCS drug. The use of a gaseous agent allowed control of duration of action simply by terminating administration. Since nitrous oxide is relatively insoluble in plasma and all body compartments, including fat, and is not metabolized, it equilibrates rapidly with brain tissue. Apart from this brief period of equilibration, the duration of action is essentially equal to the duration of inhalation. Following four pairing sessions in which 30 min. of N_2O inhalation followed saccharin drinking, the degree of CTA was directly proportional to the concentration of N_2O used. A group receiving an 80% N_2O concentration drank less after conditioning than one receiving a 60% N_2O concentration, which, in turn, showed a CTA compared to controls receiving pure oxygen. When concentration was held constant and subjects were exposed to N_2O for 0.5, 1.0, or 4.0 hours the level of CTA obtained was greater when the duration of exposure to the drug was longer.

Many of the experimental parameters which are critical in the conditioning of taste aversions have now been elucidated. Among these are the number and dose of

pretreatments and CS-UCS pairings, the choice of drugs for conditioning or pretreatments, the duration of action of these drugs at the particular doses, and the time intervals between pretreatment and conditioning and between presentation of the CS and the UCS. Because various researchers have manipulated different experimental parameters it is difficult to assess the extent to which the magnitude of the pretreatment effect depends on the specific drugs used. Part of the problem in this area has been the difficulty in choosing doses of different compounds which will produce an equivalent level of CTA. Dose-response relationships have not been thoroughly worked out for CTA in general, and are particularly scarce with respect to the pretreatment effect (Gamzu, 1974 & 1975; Goudie et al., 1976).

One of the objectives of this study was to determine the dose-response relationship for the pretreatment effect when pretreatment and training drug and dose were the same. The UCS drugs used in these studies were THC, amphetamine, diazepam, and morphine. The effect of three pretreatments on the establishment of a CTA response was determined for a given range of doses for each of these drugs. In addition, this report also presents the results of a crossover pretreatment study in which three drugs of different pharmacological class were employed. Amphetamine, diazepam, and THC each were tested as a pretreatment drug, with each agent in turn serving as the UCS training

drug. The design of this series of experiments was the same as that used in the dose-response studies except that only one particular dose of each drug was used.

CHAPTER II

MATERIALS AND METHODS

General

Subjects were naive male mice of the Swiss-Webster strain. Upon receipt the mice were allocated to home cages in the animal care facility (six mice per cage). They were allowed ad libitum access to food (Purina rat chow) and tap water for not less than one week after arrival. Lighting in this room was controlled automatically; the lights coming on at 7:00 a.m. and shutting off at 9:00 p.m. each day. At the beginning of an experiment the mice ranged from 15 to 25 g in weight. All mice used in an experiment were of the same age (three to six weeks, depending upon the experiment). Groups within an experiment did not differ with respect to mean weight or age.

Throughout most of the two week duration of an experiment the mice were restricted to a one-half hour drinking period in the morning. For this purpose the subjects were placed in individual 13 X 19 X 30 cm plexiglass cages covered on top by a metal grid. Solutions available for drinking (0.1% saccharin or tap water) were contained in 50 ml Erlenmeyer flasks fitted with a number two rubber stopper

with drinking spout. Fluid consumption was determined daily by weighing the bottles before and after the drinking session. Fluid loss due to dripping was minimized by maintaining the bottles near full volume. The bottles were filled with fresh solution at least every second day. An empty drinking spout with stopper was inserted through the bars of the cage adjacent to the spout which delivered fluid. Food was available ad libitum in both the home cages and drinking cages throughout an experiment.

An attempt was made to prevent the formation of conditioned position preferences by alternating the side of the cage in which the solutions were placed. Table I specifies the solution(s) available and the side on which they were placed on a daily basis. With the exception of the two test days (in which both solutions were available), the empty drinking spout occupied the remaining position. The position of the drinking bottle (right or left) was the same for all cages each day except for the two CTA training days. On these two days, half of the drinking bottles were on one side of the cage and half on the other.

Pretreatments were given in the home cage area on three consecutive afternoons. These injections were given four to five hours after the last groups finished drinking. Since the first groups finished drinking several hours earlier, the time between drinking and pretreatments ranged from four to seven hours for different groups. The drug

TABLE I

EXPERIMENTAL PARADIGM FOR CTA STUDIES

Day	
1	Water removed from home cage in morning.
2	One-half hour access to water on right side in morning.
3	(Pretreatment #1): One-half hour access to water on left side in morning; drug injections in afternoon.
4	(Pretreatment #2): One-half hour access to water on right side in morning; drug injections in afternoon.
5	(Pretreatment #3): One-half hour access to water on left side in morning; drug injections in afternoon.
6	One-half hour access to water on right side in morning; ad libitum access to water in home cage in afternoon.
7	Ad libitum access to water in home cage.
8	Water removed from home cage in morning.
9	(CTA Training Day #1): One-half hour access to saccharin in morning followed immediately by i.p. drug injection. The saccharin bottle is on the right for one-half of each group and on the left for the other half.
10	(CTA Training Day #2): Procedure as on day 9, except the saccharin is placed on the side opposite the previous day.
11	(Recovery): One-half hour access to water on left side in morning.
12	(Recovery): One-half hour access to water on right side in morning.
13	(Two-Bottle Choice Test #1): One-half hour access to saccharin and water in morning; saccharin is on right side and water is on left side.
14	(Two-Bottle Choice Test #2): One-half hour access to saccharin and water in morning; saccharin is on left side and water is on right side.

pretreatments were followed by a period of ad libitum access to water in an effort to regain possible weight loss due to drug effects and water deprivation.

Dose-Response Relationships for the Pretreatment Effect

Ten groups of six mice each were used in these studies. Four doses of each UCS drug were used. Four groups received both the drug pretreatments and the conditioning injections and these were labeled DD1, DD2, DD3, and DD4 (in order of increasing dosage). Another four groups received pretreatments of the drug vehicle alone, followed by conditioning injections. These were labeled VD1, VD2, VD3, and VD4. One control group received vehicle injections both for pretreatment and CTA training (VV), and another received the drug pretreatments but was injected with drug vehicle on CTA training days (DV). The dose of the drug given to group DV was the same as the highest used in any other group (DD4 and VD4). All injections were given i.p. at a constant volume of 10 ml/kg.

Delta-9 Tetrahydrocannabinol

The paradigm used in this dose-response study differed from that described in Table I. On the first two days of this study five groups (six mice each) received drug pretreatments of 1.0, 5.0, 10, 20, or 40 mg/kg Delta-9 THC

dissolved in 2% Pluronic F-68 in 0.9% saline prepared as described by Sprague and Craigmill (1976). No drug vehicle pretreatments were given. At the time of pretreatments the animals were not water deprived. Ad libitum access to water was continued until the fourth day, then the water was removed. The fifth day was the single CTA training day. After 30 minutes access to saccharin on the right side of the cage the subjects were injected with 1.0, 5.0, 10, 20, or 40 mg/kg Delta-9 THC. Each group received the same dose which had been used for pretreatments. On the following two days (days 6 and 7), the mice were given 30 minutes access to water. The water bottle was on the left side on day six and on the right side on day seven. An empty spigot occupied the adjacent position on both days. Saccharin preference was tested in a 30 minute drinking session on days eight and nine. On day eight the saccharin bottles occupied the right side of the cage for half of each group and were placed on the left side of the cage for the other half. Water bottles were placed in the remaining position for each group. On day nine the positions of all bottles were switched and subjects were again given access to both solutions for 30 minutes.

Diazepam

Drug doses of diazepam were 0.625, 1.25, 2.50, and 5.00 mg/kg. The stock solution (5 mg/ml) came dissolved in 10% propylene glycol and 2.5% ethanol. This solution

was diluted with vehicle to concentrations of 0.0625, 0.125, 0.25, and 0.50 mg/ml. The drug vehicle was a solution of 10% propylene glycol and 2.5% ethanol in physiological saline (0.9%). The volume of all injections was 10 ml/kg.

D-Amphetamine

Two independent experiments were used to cover the range of doses reported here. The first study used doses of 1.0, 2.0, 4.0, and 8.0 mg/kg d-amphetamine. Group DV received 8.0 mg/kg pretreatments in this study. Doses of 0.25, 0.50, 0.75, and 1.00 mg/kg d-amphetamine were used in the second experiment. Group DV received 1.00 mg/kg pretreatments in the second study. A 0.9% saline solution was used for vehicle injections and for dilution of the stock (0.8 mg/ml) solution such that the volume of all drug injections was 10 ml/kg.

Group DV of the first (higher doses) study was inadvertently given water prior to drinking on the second test day (day 14 of Table I). For this reason group DV received the second two-bottle choice test one day late.

A modification of the paradigm described in Table I was also necessary during the course of the second (lower doses) experiment. The room in which the mice normally were allowed the 30 minute drinking sessions was not available for two days. All groups were given fluid access in a new environment on the second CTA training day and the following recovery day (days 10 and 11 of Table I).

Morphine

Drug doses used were 3.5, 6.9, 34.6, and 69.1 mg/kg morphine sulfate. Drug vehicle injections were of a 0.9% saline solution. The saline vehicle was also used to dilute the stock (0.691 mg/ml) solution to the various concentrations desired. All injections were given i.p. in a volume of 10 ml/kg.

Demonstration of the Crossover Pretreatment Effect

Twelve groups of five mice each were used in this series of studies. Three UCS drugs of widely different pharmacological class were used in an attempt to demonstrate the crossover pretreatment effect. Diazepam (DZP) was used at a constant dose (2.5 mg/kg) for both pretreatments and CTA training injections. The drug vehicle for diazepam was a solution of 10% propylene glycol and 2.5% ethanol in physiological saline (0.9%). Delta-9 tetrahydrocannabinol (THC) was also used at a constant dose (4.0 mg/kg) for all injections. The drug vehicle for THC was a solution of 2% Pluronic F-68 in 0.9% saline as described by Sprague and Craigmill (1976). D-amphetamine (AMPH) was the third UCS drug used and was also employed at a constant dose (4.0 mg/kg) for all injections. The drug vehicle for d-amphetamine was a 0.9% saline solution. All drug and vehicle injections were given i.p. in a volume of 10 ml/kg.

Groups were labeled according to the pretreatment drug or vehicle used and the CTA training drug used. Thus, group AMPH/DZP received d-amphetamine pretreatments prior to CTA training with diazepam. Six groups (AMPH/DZP, THC/DZP, AMPH/THC, DZP/THC, DZP/AMPH, and THC/AMPH) were included in the first experiment. A second experiment included groups THC/THC, AMPH/AMPH, and DZP/DZP; and a third experiment included the control groups VEH/THC, VEH/DZP, and VEH/AMPH (VEH stood for drug vehicle and depended upon the CTA training drug with which it was matched).

Procedures for this series of studies differed somewhat from those described in Table I. The mice were housed in large (16 X 40 X 33 cm) plexiglass cages covered by a metal framework. Five mice were kept in each cage. The drinking cages were the same as described previously. Drinking was monitored in the experimenter's lab area during the first two experiments but occurred in a different room during the third experiment. A full hour of fluid access was allowed on day two of each experiment instead of the 30 minutes specified in Table I. During the third (control) experiment all subjects received saccharin on the same side of the cage on CTA training days. Saccharin bottles were on the left for all cages on day 9 and on the right on day 10 (see Table I).

Statistical Analysis

In all experiments the saccharin preference ratio

(SPR) scores were averaged over the two test days to obtain a single score for each subject. These derived scores were then averaged to obtain a mean SPR score and standard deviation for each group.

Student's one-tailed t test for unpaired observations was the principal test criterion throughout the dose-response experiments. The level of significance for all t tests (one and two-tailed) was set at $p \leq 0.05$. Exceptions to this procedure were made for comparisons between experiments and for the treatment of group DV. A comparison of scores for group VV across all experiments and for group DV between the two experiments dealing with d-amphetamine employed a two-tailed t test. Within each experiment, group DV was compared to group VV using a two-tailed t test and was compared to group DD4 using a one-tailed t test (H_0: DV-DD4=0; H_A: DV-DD4 > 0). Comparisons among the means of other groups fell into three categories. Each drug pretreated group was compared to the corresponding vehicle pretreated group of the same dose to test for the pretreatment effect. All groups other than DV were compared to group VV using a one-tailed t test. Finally, a one-tailed t test was used for comparisons among groups which received the same treatment regimen but differed in dose. These tests were conducted with the expectation that the group which received the lowest dose would obtain the highest SPR.

Student's two-tailed t test for unpaired observations was used for comparisons among experimental groups in the crossover pretreatment experiments. However, each experimental group was compared to the corresponding vehicle pretreated control group using a one-tailed t test. The level of significance for all t tests (one and two-tailed) was set at $p \leq 0.05$. Comparisons among groups were made as if all had been included in a single experiment.

CHAPTER III

RESULTS

Dose-Response Relationships for the Pretreatment Effect

Delta-9 Tetrahydrocannabinol

Figure 1 shows the mean saccharin preference ratio (SPR) and standard deviation for each dose group. Groups DD3 (10 mg/kg), DD4 (20 mg/kg), and DD5 (40 mg/kg) each had a significantly lower SPR than group DD1 (1.0 mg/kg). In addition, groups DD3 (10 mg/kg) and DD4 (20 mg/kg) each had a significantly lower SPR than group DD2 (5.0 mg/kg).

Diazepam

Figure 2 presents the mean SPR for each group. Table III (see Appendix) lists the mean SPR and standard deviation for each group. Group DD3 (2.5 mg/kg) had a significantly higher SPR than did group VD3 (2.5 mg/kg). Groups DD1 (0.625 mg/kg), VD2 (1.25 mg/kg), VD3 (2.5 mg/kg), and VD4 (5.0 mg/kg) each had a lower SPR than group VV. Groups VD2 (1.25 mg/kg), VD3 (2.5 mg/kg), and VD4 (5.0 mg/kg) also had a lower SPR than group VD1 (0.625 mg/kg). Group DV (5.0 mg/kg) did not differ from group VV or from group DD4 (5.0 mg/kg).

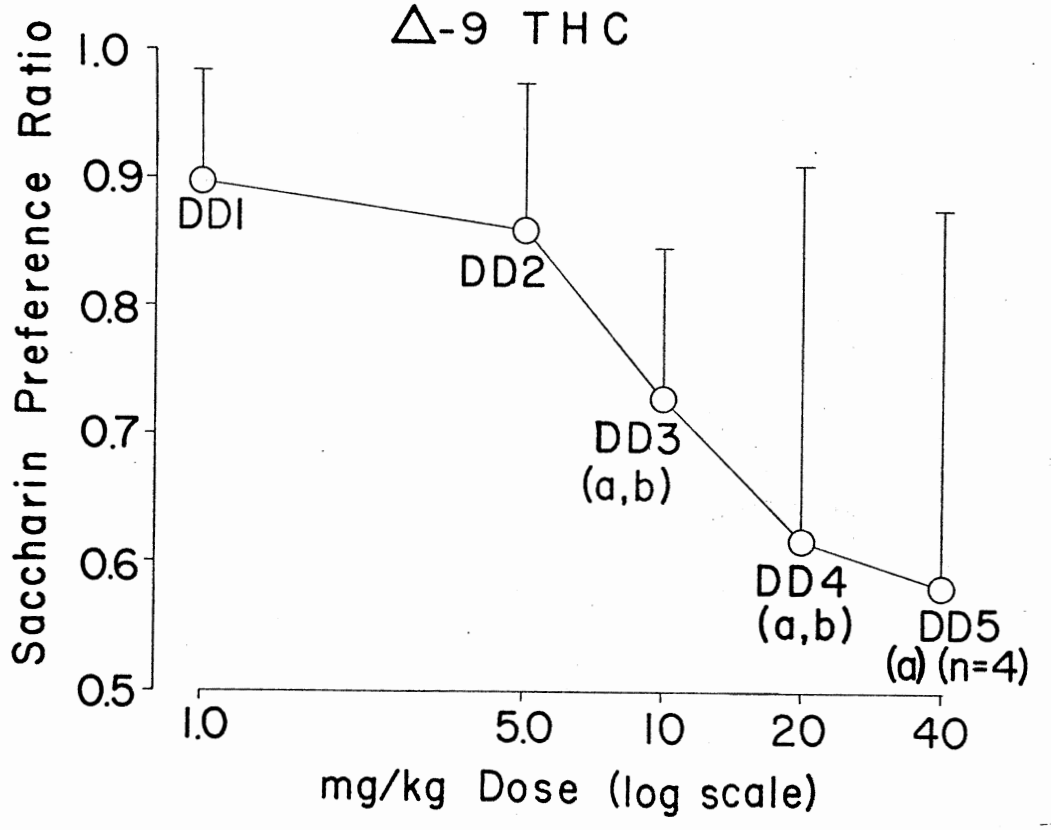

Figure 1. Dose-Response Relationship for Delta-9 THC. Two pretreatments and one training injection of the drug were given to each group of six male mice. (a) indicates a significant difference between group DD1 and any other group ($p < 0.05$). (b) indicates a significant difference between group DD2 and any other group ($p < 0.05$).

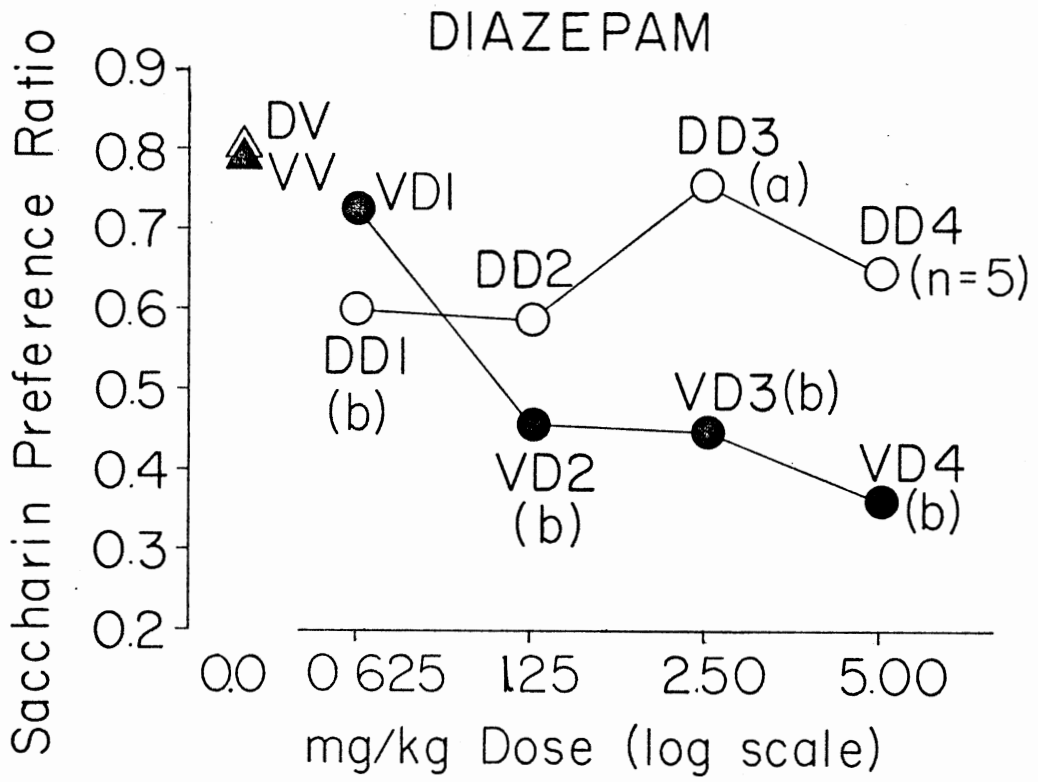

Figure 2. Dose-Response Relationship for Diazepam. Three pretreatments and two training injections of the drug or its vehicle were given to each group of six male mice. Drug pretreated groups are designated by open points and vehicle pretreated groups by solid points. (a) indicates a significant difference between DD and VD groups ($p < 0.05$). (b) indicates a significant difference between group VV and any other group ($p < 0.05$).

D-Amphetamine

Figure 3 presents the mean SPR scores graphically and Table IV (see Appendix) lists the mean SPR and standard deviations for each group in these two experiments. Three groups were represented in both experiments. This replication allowed a comparison of scores for these groups between the two studies. Group VV of experiment one (high doses) did not differ from group VV of experiment two (low doses) when tested with a two-tailed Student's t test. Likewise, group DD1 of experiment one (1.0 mg/kg) did not differ from group DD4 of experiment two (1.0 mg/kg). Finally, group VD1 of experiment one (1.0 mg/kg) did not differ from group VD4 of experiment two (1.0 mg/kg).

In experiment one (high doses), group DD3 (4.0 mg/kg) had a significantly higher SPR than group VD3 (4.0 mg/kg). All groups except DD1 (1.0 mg/kg) and DV (8.0 mg/kg) had a significantly lower SPR than group VV. Within treatment conditions, group DD4 (8.0 mg/kg) had a lower SPR than groups DD1 (1.0 mg/kg), DD2 (2.0 mg/kg), or DD3 (4.0 mg/kg) and group VD3 (4.0 mg/kg) had a lower SPR than group VD1 (1.0 mg/kg). Group DV (8.0 mg/kg) did not differ from group VV but had a significantly higher SPR than group DD4 (8.0 mg/kg).

In experiment two (low doses), group DD1 (0.25 mg/kg) had a significantly higher SPR than group VD1 (0.25 mg/kg).

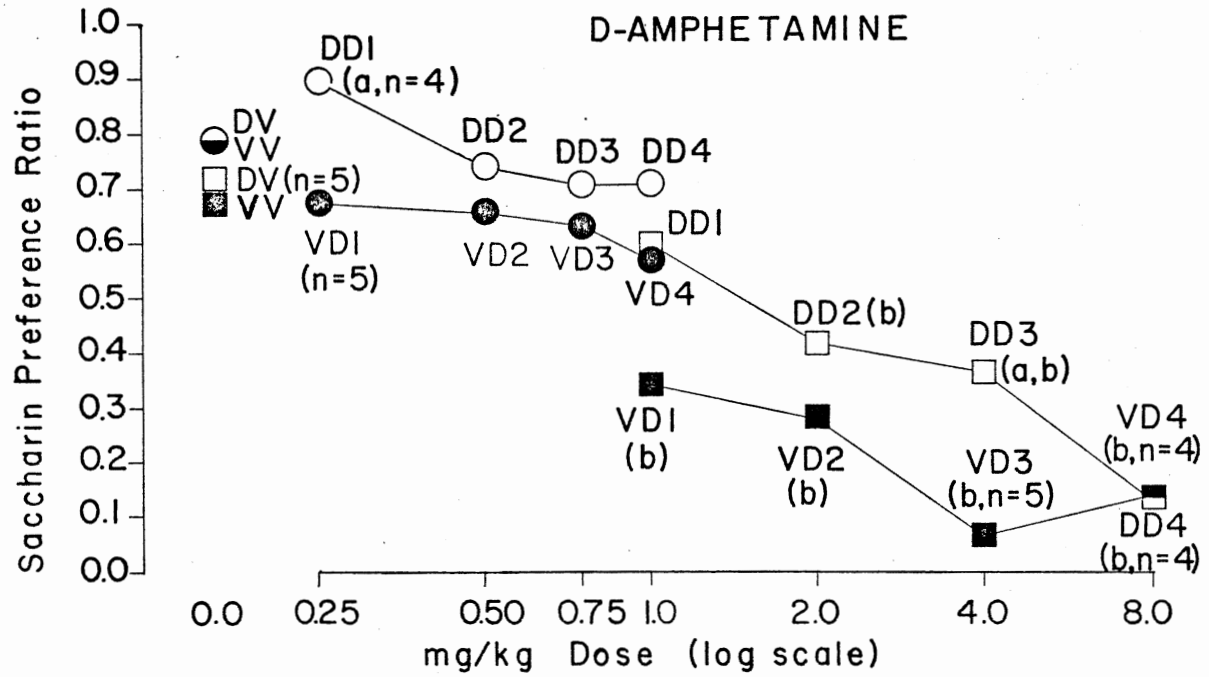

Figure 3. Dose-Response Relationship for D-Amphetamine. Three pretreatments and two training injections of the drug or its vehicle were given to each group of six male mice. Drug pretreated groups are designated by open points and vehicle pretreated groups by solid points. (a) indicates a significant difference between DD and VD groups ($p < 0.05$). (b) indicates a significant difference between group VV and any other group ($p < 0.05$). Control groups DV and VV are marked with circles for the lower doses and squares for the higher doses.

Group VV did not differ from any other group. Within treatment conditions, groups DD2 (0.50 mg/kg) and DD3 (0.75 mg/kg) had a lower SPR than group DD1 (0.25 mg/kg). Group DV (1.0 mg/kg) did not differ from group VV or group DD4 (1.0 mg/kg).

Morphine

Figure 4 presents the mean SPR for each group. Table V (see Appendix) lists the mean SPR and standard deviation for each group. Group DD4 (69.1 mg/kg) had a higher SPR than group VD4 (69.1 mg/kg). Groups VD3 (34.6 mg/kg), VD4 (69.1 mg/kg), DD2 (6.91 mg/kg), DD3 (34.6 mg/kg), and DD4 (69.1 mg/kg) each had a lower SPR than group VV. Within treatment conditions, groups VD3 (34.6 mg/kg) and VD4 (69.1 mg/kg) each had a lower SPR than both group VD1 (3.46 mg/kg) and group VD2 (6.91 mg/kg). Group DV (69.1 mg/kg) did not differ from group VV but had a higher SPR than group DD4 (69.1 mg/kg).

A two-tailed t test was used to compare mean SPR scores for group VV between each of the four experiments which included this group (diazepam, amphetamine, and morphine). No differences were found in the SPR of group VV between any of the experiments.

Demonstration of the Crossover Pretreatment Effect

Table II presents the mean SPR and standard deviation

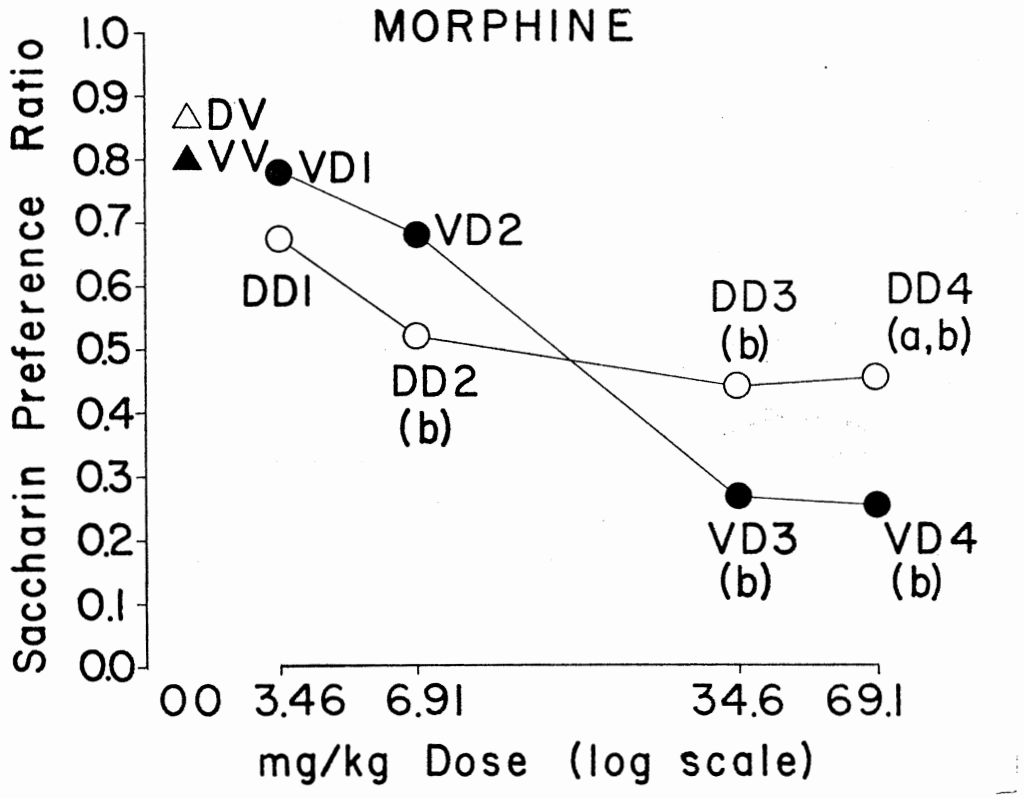

Figure 4. Dose-Response Relationship for Morphine. Three pretreatments and two training injections of the drug or its vehicle were given to each group of six male mice. Drug pretreated groups are designated by open points and vehicle pretreated groups by solid points. (a) indicates a significant difference between DD and VD groups ($p < 0.05$). (b) indicates a significant difference between group VV and any other group ($p < 0.05$).

TABLE II

MEAN SACCHARIN PREFERENCE RATIOS IN CROSS-OVER PRETREATMENT EXPERIMENTS

Pretreatment Drug	CTA Training Drug		
	Diazepam (2.5 mg/kg)	Amphetamine (4.0 mg/kg)	Delta-9 THC (4.0 mg/kg)
Diazepam (2.5 mg/kg)	*0.784 ± 0.092	0.313 ± 0.153	*0.740 ± 0.103
Amphetamine (4.0 mg/kg)	*0.681 ± 0.097	*0.601 ± 0.153	0.713 ± 0.117
Delta-9 THC (4.0 mg/kg)	0.252 ± 0.172	0.370 ± 0.210	0.749 ± 0.244
Vehicle	**0.300 ± 0.246	0.167 ± 0.205	0.478 ± 0.277

*Significant pretreatment effect.

**N=4 for this group; N=5 for all other groups.

for each group. When all the pretreatments were of the appropriate vehicle there were no differences between any of the three vehicle pretreated groups. Groups DZP/DZP and AMPH/DZP both had a significantly higher SPR than group VEH/DZP. Group AMPH/AMPH had a higher SPR than group VEH/AMPH. Finally, group DZP/THC had a significantly higher SPR than group VEH/THC.

No combination of different pretreatment and training drugs showed symmetrical crossover. Group AMPH/DZP had a significantly higher SPR than group DZP/AMPH. Group DZP/THC had a significantly higher SPR than group THC/DZP. Group AMPH/THC had a significantly higher SPR than group THC/AMPH.

When the CTA training drug was diazepam, groups DZP/DZP and AMPH/DZP both had a significantly higher SPR than group THC/DZP. When the CTA training drug was d-amphetamine, group AMPH/AMPH had a significantly higher SPR than group DZP/AMPH. When THC was the CTA training drug, no two drug pretreated groups differed from each other.

When the pretreatment drug was diazepam, groups DZP/DZP and DZP/THC each had a significantly higher SPR than group DZP/AMPH. When the pretreatment drug was d-amphetamine, no difference was found among the three CTA training drugs. When the pretreatment drug was THC, groups THC/DZP and THC/AMPH each had a significantly lower SPR than group THC/THC.

A two-tailed t test was used to compare certain groups of this experiment to groups in the dose-response experiments which received the same treatment. Group VEH/DZP (2.5 mg/kg) did not differ from group VD3 (2.5 mg/kg) of the diazepam dose-response experiment and group DZP/DZP did not differ from group DD3 (2.5 mg/kg) of the diazepam dose-response experiment. Likewise, group VEH/AMPH (4.0 mg/kg) did not differ from group VD 3 (high dose, 4.0 mg/kg) of the amphetamine dose-response experiment and group AMPH/AMPH did not differ from group DD3 (high dose, 4.0 mg/kg) of the amphetamine dose-response experiment.

CHAPTER IV

DISCUSSION

Choice of Paradigm

When a two-bottle choice test is used to determine taste preference it is necessary to control for the possibility that a subject may form a position preference. In such a case an animal prefers to drink from a spout located in a particular part of the cage, regardless of the fluid available through this spout (Myers & Veale, 1972). The alternation of sides on which the fluid filled bottle was placed (Table I) was designed to prevent the formation of such position preferences. The use of two CTA training days allowed the mice to taste saccharin once on each side of the cage as well as having the effect of strengthening conditioning. Two recovery days followed the CTA training trials. Thus, the last CTA training day and the first two-bottle choice test day were separated by a 36 hour interval which was felt to be sufficient for most drug effects to wear off prior to the first preference test. The two-bottle preference test was conducted twice so that position preferences (indicated by a large change in saccharin preference ratio) could be checked. Because no drug injections followed

saccharin drinking on test days, these may actually be considered extinction trials. Saccharin preference scores on test day #2 could be influenced by extinction learning on test day #1.

A rigorous statistical test for position preferences would require an analysis of variance in which the two test days represent repeated sampling of an experimental unit. Such a procedure rarely finds position preferences (subsample number) to be a significant factor in the determination of scores when bottle positions have been alternated as described (Fischer, 1978). In lieu of such an analysis the author simply recorded the difference in scores between the two test days for individual subjects. A change in saccharin preference ratio of 0.5 or above was arbitrarily considered large enough to suspect a position preference. Over all experiments only 5% of subjects had a shift in preference ratio of 0.5 or more.

For the purpose of graphical representation and the calculation of group scores, an individual's preference ratio score was averaged over both test days. This procedure yielded a value of n which was equivalent to the number of subjects X number of test days. Such a procedure also obscures the effect of position preferences in the rare individual which may exhibit this behavior, since a high score on test day #1 and a low score on test day #2 yields an overall score near 0.5, which indicates equal preference for both solutions.

Although one pretreatment is often sufficient to attenuate CTA formation (Cannon et al., 1975), the degree of attenuation appears to be proportional to the number of pretreatments given (Goudie et al., 1976). Previous work indicated that three to five pretreatments are effective with a wide variety of UCS drugs and experimental paradigms (see Chapter I). Three pretreatments were chosen as the minimum number which might be effective with various drugs and doses without producing high mortality in the higher dose groups. Cappell and LeBlanc (1975a) indicate that CS-UCS pairing must follow pretreatments within seven days in the paradigm they employed. Yet, if the CTA training followed too closely after the pretreatments the subjects might be learning under the influence of the drugs. The interval of 3.5 days between pretreatments and CTA training was thought to be long enough for drug effects to wear off and weight to be regained, without jeopardizing the effectiveness of the pretreatments.

Pretreatments were given well after the drinking sessions and in the home cage area, but CTA training injections were given immediately after drinking and in the drinking cage area. The "association hypothesis" for CTA claims that subjects may form aversions to handling, the injection procedure, or other stimuli in the environment (Gamzu, 1977). The inability of many potential stimuli to act as the CS in CTA training has been discussed in

Chapter I. Though the issue of the "equipotentiality of conditioned stimuli" is not yet fully resolved, there is much evidence that at least the area in which injections are given does not affect the acquisition or attenuation of a CTA response (Jacquet, 1973; Cannon et al., 1975). Even when subjects were pretreated in quite different environments (some groups placed in cages rubbed with oil of cloves immediately before and after injections) the degree of attenuation achieved through pretreatment did not differ (Stewart & Eikelboom, 1978).

Dose-Response Relationships for the Pretreatment Effect

Delta-9 Tetrahydrocannabinol

This experiment was a pilot study and was conducted before the paradigm described in Table I was settled upon. This difference in the paradigm and the absence of control groups VD1-4, VV, and DV limit the extent to which these results can be compared to results for other UCS drugs. The conclusion from this experiment is that for Δ-9 THC the saccharin preference ratio scores of pretreated animals decrease with increasing dose. Without the vehicle pretreated controls it is not possible to make any statements about how the degree of attenuation of CTA may vary with increasing dose. Most dose-response studies of CTA in drug naive (not pretreated) animals have shown these

preference ratio scores also decrease with increasing dose. This trend has been observed for non-pretreated animals when delta-9 THC is the UCS drug (Craigmill, 1978). The data indicates that such subjects have preference ratio scores below 0.5 at the doses used here. Curves for both the pretreated and drug naive animals thus show the same pattern of decreasing scores with increasing dose. It is not known whether these two curves would maintain a constant distance between each other. Figure 1 suggests that the scores of pretreated animals are roughly constant at doses below 5.0 mg/kg or above 40 mg/kg. If the curve for drug naive animals showed the same inflection points (maintaining a constant distance between the curves) this would indicate that the magnitude of attenuation of CTA due to pretreatment is independent of dose of THC. Further research with this drug should include both drug pretreated and vehicle pretreated animals in the same experiment and should inlcude doses above 40 mg/kg.

Diazepam

The pretreatment effect was demonstrated only for the 2.50 mg/kg dose of diazepam (groups DD3 vs. VD3). CTA training in vehicle pretreated groups produced an aversion at doses of 1.25, 2.50, and 5.00 mg/kg as reflected in the difference between these groups and group VV. An examination of the actual scores for these groups reveals that the

aversion was a mild one since preference scores were not far below the 0.5 (equal preference) mark. The pretreatment effect would be harder to demonstrate at doses which produce only mild aversions and this factor may account for the finding that pretreatment only produced an attenuation of CTA at one dose (2.50 mg/kg). Groups VD2, VD3, and VD4 did not differ from each other although each differed from VD1. Thus, the minimum effective dose of diazepam for producing CTA in this paradigm appears to be between 0.625 and 1.25 mg/kg. Group DD1 (0.625 mg/kg) also had a significantly lower SPR than group VV, although group VD1 (0.625 mg/kg) did not differ from VV. There is no statistical basis for concluding that group DD1 had a lower saccharin preference ratio than group VD1. Such a hypothesis is excluded by the use of a one-tailed test, and the appearance that the two curves cross in Figure 2 cannot be statistically validated. In any case, the research hypothesis specifically excludes the possibility that a drug pretreated group may have a lower SPR (have a stronger aversion) than the corresponding vehicle pretreated group. The use of a one-tailed test was based upon evidence provided by previous research on the pretreatment effect (see Chapter I).

Group DV (5.0 mg/kg) did not differ from group VV, which shows that pretreatment per se did not influence the establishment of CTA. Group DV received the highest

dose used in this study (5.0 mg/kg) and it is assumed that the use of lower doses would not have produced any difference between group DV and group VV.

D-Amphetamine

The paradigm outlined in Table I was slightly modified for the second (low doses) experiment with this drug. The 1.0 mg/kg dose was included in both experiments and neither the drug pretreated (DD) nor vehicle pretreated (VD) groups differed in saccharin preference ratios between the two experiments. The SPR scores of group VV also did not differ between the two experiments. The procedural differences between the two experiments did not produce any statistically reliable difference in preference ratios, although Figure 3 suggests the trend may have been for the 1.0 mg/kg groups to score somewhat higher in the second (low doses) study.

The pretreatment effect was demonstrated at doses of 0.25 and 4.0 mg/kg d-amphetamine. CTA training in vehicle pretreated subjects produced aversions at doses of 1.0, 2.0, 4.0, and 8.0 mg/kg as reflected in the difference between these groups and group VV. These aversions were fairly strong (SPR < 0.35), yet pretreatment was only effective at two doses. At a dose of 4.0 mg/kg, pretreatments attenuated but did not entirely block CTA (group DD3 of part one had an SPR below group VV but above group VD3).

At a dose of 0.25 mg/kg the pretreatments entirely blocked CTA formation, but at this dose little aversion could be produced to begin with. Although Figure 3 suggests that group DD1 (0.25 mg/kg) had a higher SPR than group VV, the test hypothesis as written forces the conclusion that this group did not differ from group VV.

It would appear unlikely that doses of 0.25 and 4.0 mg/kg could produce the pretreatment effect when all doses in between were ineffective. A more likely alternative is that the 0.25 mg/kg dose does not actually produce attenuation of CTA and the results reported here were due to chance at this dose. Group DD1 (0.25 mg/kg) had one of the lowest variances reported in any experiment (as well as a low value of n=4) which contributed to the finding that CTA was attenuated.

The shape of the two curves in Figure 3 suggests that the magnitude of the pretreatment effect is independent of dose within a certain range. It appears that the degree of attenuation may be constant between doses of 0.50 and 4.0 mg/kg d-amphetamine. No attenuation of CTA occurred at a dose of 8.0 mg/kg and probably none would be found at doses below 0.25 mg/kg, but further work is needed to confirm this point.

Group DV (1.0 and 8.0 mg/kg) did not differ from group VV in either experiment. Thus, pretreatment per se did not influence the establishment of CTA. It is assumed

that the use of other doses for group DV would not have produced any difference between this group and group VV, since the highest dose (8.0 mg/kg) did not do so.

Morphine

The pretreatment effect was demonstrated only for the highest dose (69.1 mg/kg) of morphine. CTA training in vehicle pretreated groups produced an aversion at doses of 34.6 and 69.1 mg/kg as reflected in the difference between these groups and group VV. These aversions were fairly strong (SPR < 0.30) and the attenuation produced by pretreatment was not total (the group pretreated with 69.1 mg/kg morphine had an SPR higher than the vehicle pretreated group but lower than group VV).

The appearance that the two curves cross in Figure 4 cannot be statistically validated. The use of a one-tailed test specifically excludes the possibility that a drug pretreated group may have a lower SPR than the corresponding vehicle pretreated group. The use of a one-tailed test was based on evidence provided by previous research on the pretreatment effect (see Chapter I).

Group DV (69.1 mg/kg) did not differ from group VV, which shows that pretreatment per se did not influence the establishment of CTA. Group DV received the highest dose used in this experiment and it is assumed that the use of lower doses would not have produced any difference between group DV and group VV.

Demonstration of the Crossover Pretreatment Effect

The demonstration of a crossover pretreatment effect requires that the doses of all UCS drugs employed be equally effective in producing CTA. Doses should also be chosen which are known to produce attenuation of CTA when same-drug pretreatments are given. Ideally, the magnitude of attenuation produced by same-drug pretreatments should be equivalent for each drug at the doses used.

No significant differences in saccharin preference ratio were detected among the three vehicle pretreated groups (Table II). This result indicates that diazepam (2.5 mg/kg), amphetamine (4.0 mg/kg), and THC (4.0 mg/kg) were equally effective UCS drugs at the doses used. It should be noted that each of the pretreated groups had a high variance.

Diazepam pretreatment attenuated CTA produced by diazepam, and amphetamine pretreatment attenuated CTA produced by amphetamine. However, THC pretreatment did not attenuate CTA produced by THC. These results replicate the findings from the dose-response studies which show that a 2.5 mg/kg dose of diazepam and a 4.0 mg/kg dose of amphetamine each are capable of attenuating CTA produced by the same drug. When diazepam or amphetamine were the UCS drugs the mean SPR of both the vehicle pretreated and drug pretreated groups did not differ between the two experiments.

For these two drugs the degree of attenuation produced by pretreatment was the same in both experiments.

The failure to demonstrate the pretreatment effect for THC places a limit on conclusions concerning the effect of THC pretreatments on CTA induced by diazepam or amphetamine. Since THC pretreatments failed to attenuate CTA induced by THC it is not surprising that THC pretreatments did not attenuate CTA induced by diazepam or amphetamine. Diazepam pretreatments did attenuate CTA induced by THC (group DZP/THC) and it is interesting to note that the mean SPR of this group was quite close to the mean for group THC/THC. The high variance of group THC/THC was due to only one subject (which had an SPR of 0.32, while the other four ranged from 0.81 to 0.89) and this effect may well have obscured a pretreatment effect for group THC/THC.

Groups AMPH/DZP and DZP/THC demonstrated the crossover pretreatment effect. Tests for the symmetry of crossover are only valid between combinations of diazepam and amphetamine since group THC/THC did not have an attenuated CTA. Pretreatment with diazepam or amphetamine attenuated CTA induced by the same compound. Amphetamine pretreatment attenuated CTA induced by diazepam, but diazepam pretreatment failed to attenuate CTA induced by amphetamine.

Conclusions

Results of the research on CTA present a challenge to both the psychologist and pharmacologist. Behaviorists

have traditionally regarded all stimuli as neutral, positively reinforcing, or negatively reinforcing. It has been demonstrated that the same drug stimuli that are effective in maintaining self-administration are also capable of inducing CTA (Cappell & LeBlanc, 1977). This indication that the same dose of a drug may be either positively or negatively reinforcing receives additional support from the finding that both behaviors (self-administration and taste aversion) may occur simultaneously (Wise et al., 1976). Conditioned taste aversion also differs from classical conditioning with respect to the "contiguity rule" of conditioning and the rule of the "equipotentiality of conditioned stimuli" and consequently has not been well incorporated into general learning theory (see Chapter I).

Some resolution of the apparent paradoxes of CTA is obtained by examining these phenomena from a viewpoint closer than the organismic level. One of the tenets of pharmacology is that drugs have multiple actions. Diazepam is used as a minor tranquilizer, a muscle relaxant, and an anticonvulsant. The view that a drug must be either positively or negatively reinforcing (or neutral) depends upon the assumption that the drug effects act as a single stimulus. Cappell and LeBlanc (1973) note that reinforcement schedules and ongoing behavior at the time of conditioning have much influence on the consequences of

reinforcers, thus the nature of a drug's reinforcing action may be situation-specific.

Tolerance and/or dependence have often been implicated in the pretreatment effect for CTA. Kalant at al. (1971) define tolerance as an acquired change due to repeated exposure to a drug such that

> . . . an increased amount of drug is required to produce the same specified degree of effect, or less effect is produced by the same dose of drug. This definition of tolerance is valid only for a specified individual drug action, and not necessarily for the composite picture of all actions of a given drug on the whole organism (p. 137).

Tolerance is further divided into two classes. Dispositional tolerance includes changes in a drug's absorption, distribution, excretion, and metabolism which alter its effect upon the target tissue. Functional tolerance includes changes in the target tissue which make it less sensitive to the drug. The concentration of the drug in the blood or brain (rather than the administered dose) is needed to differentiate these two classes of tolerance. Another common distinction is between physiological and psychological (or "learned") tolerance. The former is said to involve homeostatic control mechanisms while the latter involves the learning of new skills and adaptive behaviors to circumvent the effects of a drug. However, Kalent et al. (1971, p. 158), in a review of the research on this question, conclude that ". . . 'learned tolerance' is essentially the same as 'physiological tolerance,' except

that it is acquired somewhat more rapidly." They propose the term "behaviorally augmented tolerance" in place of "learned tolerance" to emphasize the similarity to physiological tolerance.

Cross-tolerance between ethanol and barbiturates has long been recognized. The metabolism of many compounds, including psychoactive agents, barbiturates, other hypnotics, sedatives, tranquilizers, and antihistamines, is known to increase rapidly through induction of drug-metabolizing systems. More than 200 drugs and environmental agents are known to stimulate the activity of drug-metabolizing enzymes. In particular, the hepatic microsomal mixed-function oxidase system has a low order of substrate specificity and its induction by one drug may increase the rate of metabolism of many other drugs (metabolic cross-tolerance). The fact that drug X can induce the metabolism of drug Y does not imply that the converse is true. A drug may also be capable of inducing the metabolism of other compounds even though it does not induce its own metabolism. For example, chlorpromazine induces hepatic microsomal metabolism of other drugs and its metabolism is induced by phenobarbital pretreatment, yet chlorpromazine does not cause significant induction of its own metabolic enzymes (Kalant et al., 1971).

The results of the experiments reported here indicate that the pretreatment effect is operative for certain doses

of diazepam (2.5 mg/kg), amphetamine (4.0 mg/kg), and morphine (69.1 mg/kg). In a crossover pretreatment design the same two doses of diazepam and amphetamine produced equivalent levels of aversion in vehicle pretreated controls and the pretreatment effect was again demonstrated at these doses. A crossover pretreatment effect was demonstrated for subjects pretreated with amphetamine (4.0 mg/kg) and conditioned with diazepam (2.5 mg/kg). However, diazepam pretreatment did not attenuate CTA induced by amphetamine and therefore symmetry of the crossover pretreatment effect was not demonstrated. The pretreatment effect is interpreted in terms of tolerance due to the repeated exposures to the drug prior to conditioning. Cross-tolerance is suggested as a possible explanation for the crossover pretreatment effect, with the notation that the induction of such tolerance was not symmetrical. These hypotheses are offered without reference to the class of tolerance involved or the cellular mechanism of tolerance.

One objection to the tolerance hypothesis is that it is difficult to explain the extremely wide range of effective drugs using this concept. The induction of tolerance and cross-tolerance for such a wide range of drugs has not been established. Since agonist-antagonist pairs are often effective it would be necessary to show that more antagonists have agonist activity than has heretofore been demonstrated.

Another hypothesis which overcomes the difficulty of explaining the wide range of effective drugs is the stress hypothesis. According to this hypothesis, certain physiological changes which occur under the influence of drug induced stress form the aversive properties common to CTA regardless of the specific UCS (Fischer, 1978). The pretreatment and crossover pretreatment effects are due to adaptation and/or habituation to these as yet unspecified physiological changes. The stress hypothesis and the tolerance hypothesis need not be mutually exclusive.

The generality of the stress and tolerance hypotheses is both an advantage and a disadvantage. Only a very general phenomenon could explain the wide range of effective UCS drugs and the apparent paradoxes of CTA. But models for the fundamental cellular mechanism of tolerance or stress are still rudimentary. A few physiological processes known as "biochemical correlates" of tolerance have been established but whether they are causes or consequences of tolerance has not been determined (Kalant et al., 1971). As stated here, the stress and tolerance hypotheses are hardly specific enough to allow for refutation and more knowledge of the details of these two phenomena are needed. The difficulties encountered with these two hypotheses are certainly no greater than those encountered with the alternatives. Perhaps Cappell and LeBlanc (1975a, p. 161) were correct in speculating that "Very likely, no single hypothesis will be able to embrace all of the data in this general area. . . ."

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APPENDIX

TABLES III, IV, AND V

TABLE III

GROUP SCORES FOR DIAZEPAM
DOSE-RESPONSE STUDY

Group	Dose (mg/kg)	n	Mean Saccharin Preference Ratio	Standard Deviation
DD1	0.625	6	0.599	0.103
VD1	0.625	6	0.728	0.152
DD2	1.25	6	0.586	0.292
VD2	1.25	6	0.455	0.322
DD3	2.50	6	0.754	0.102
VD3	2.50	6	0.441	0.352
DD4	5.00	5	0.648	0.264
VD4	5.00	6	0.361	0.276
DV	5.00	6	0.802	0.101
VV	0.00	6	0.792	0.115

TABLE IV

GROUP SCORES FOR D-AMPHETAMINE
DOSE-RESPONSE STUDY

Group	Dose (mg/kg)	n	Mean Saccharin Preference Ratio	Standard Deviation
		Part 1: High Doses		
DD1	1.00	6	0.601	0.262
VD1	1.00	6	0.343	0.342
DD2	2.00	6	0.418	0.248
VD2	2.00	6	0.279	0.371
DD3	4.00	6	0.364	0.209
VD3	4.00	5	0.068	0.113
DD4	8.00	4	0.129	0.104
VD4	8.00	4	0.137	0.237
DV	8.00	5	0.717	0.251
VV	0.00	6	0.672	0.159

TABLE IV (Continued)

Group	Dose (mg/kg)	n	Mean Saccharin Preference Ratio	Standard Deviation
		Part 2: Low Doses		
DD1	0.25	4	0.897	0.029
VD1	0.25	5	0.076	0.218
DD2	0.50	6	0.740	0.176
VD2	0.50	6	0.658	0.131
DD3	0.75	6	0.709	0.100
VD3	0.75	6	0.633	0.299
DD4	1.00	6	0.709	0.275
VD4	1.00	6	0.570	0.303
DV	1.00	6	0.799	0.201
VV	0.00	6	0.776	0.141

TABLE V

GROUP SCORES FOR MORPHINE
DOSE-RESPONSE STUDY

Group	Dose (mg/kg)	n	Mean Saccharin Preference Ratio	Standard Deviation
DD1	3.46	6	0.674	0.276
VD1	3.46	6	0.781	0.109
DD2	6.91	6	0.518	0.192
VD2	6.91	6	0.679	0.350
DD3	34.6	6	0.438	0.266
VD3	34.6	6	0.262	0.087
DD4	69.1	6	0.449	0.212
VD4	69.1	6	0.250	0.141
DV	69.1	6	0.868	0.078
VV	0.00	6	0.797	0.094

VITA[2]

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